— Regular Article —

# Korean Red Ginseng Extract Suppresses the Progression of Alcoholic Fatty Liver in a Rat Model

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Alcoholic fatty liver (AFL) is the most common liver disease among Korean men, and Korean red ginseng has been used as a folk medicine to diverse diseases in Korea. Therefore, we examined if Korean red ginseng extract (KRG) could be a suppressive agent on AFL in a rat model or not. Experimental rats were fed the Lieber DeCarli diet with 36% of energy intake from ethanol, and divided into three groups which daily co-administered KRG 0, 700 and 1400 mg/kg for six weeks, respectively. Naive rats were fed iso-caloric control diet without ethanol and KRG. We investigated histopathological hepatic characteristics, hepatic and plasma lipid concentrations, hepatic hydroxyproline contents, heart/liver radioactivity ratio of $^{201}$Thallium and liver/body weight of the rats at the end point. Ethanol intake brought about steatotic, inflammatory, necrotic and fibrotic changes of livers significantly, and it also lead the rats to increase hepatic triglyceride and hydroxyproline contents, plasma total cholesterol and low density lipoprotein cholesterol levels, and liver/body weight. However, co-administration of KRG 1400 mg/kg suppressed fat accumulation and fibrotic initiation in AFL rat model significantly. It was also inclined to attenuate inflammatory cell infiltration, hydroxyproline accumulation, and increasing liver/body weight, even though plasma lipid levels and heart/liver ratios were not successfully improved by six-week-long intakes of KRG. In conclusions, co-administration of KRG 1400 mg/kg could significantly suppress steatosis in AFL rat model, and it might need longer ingestion of KRG than six weeks to improve plasma lipid imbalance.

**Key words** —— Korean red ginseng, alcoholic fatty liver, fibrosis

## INTRODUCTION

In the East Asian countries including China, Japan and South Korea, various folk remedies have been prescribed for the treatment of liver diseases. Researches are ongoing to define the preventive and curative properties of these natural materials such as Hoveniadulcis,[1] Plantagoasiatica L. seeds,[2] and the fruits of Chrysanthemum indicum Linné,[3] Meliatoosendan fruit,[4] and Schisandrachinensis Baill.[5] Above all, Korean ginseng, the root of *Panax ginseng*, has been prescribed as the most precious folk medicine in many oriental nations. Therapeutic effects of Korean ginseng have been shown in a lot of studies on hyperlipidemia,[6] tumors and cancers,[7] oxidative stress,[8] arthrosclerosis,[9] hypertension,[10] diabetes,[11] and liver toxicity.[12] The steamed and dried form of Korean ginseng, known as Korean red ginseng, is less-toxic, preserved longer, and has different bioactivities when compared to fresh Korean ginseng.[13, 14] Total saponin contents including panaxadiol and panaxatriol are also increased in Korean red ginseng,[13–15] which have demonstrated protective effects on hepatotoxicity induced by carbon tetrachloride ($CCl_4$), acetaminophen, and high fat diets in animal studies.[12, 16, 17] Due to these benefits on hepatotoxicity of Korean red ginseng, it was considered to apply to alcohol-induced hepatotoxicity such as alcoholic fatty liver (AFL), the most prevalent disease among over 55% of Korean men aged from forty to sixty years old.[18] AFL is the initial stage of liver cirrhosis related to heavy alcohol consumption,[19] and the subsequent onset of alcohol-induced liver diseases is the fourth leading cause of death following

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malignant carcinomas, cerebro-/cardiovascular diseases and respiratory diseases. It is also a cause of significant morbidity before death, and a major expense to the healthcare system in Republic of Korea.[20] Therefore, we examined if Korean red ginseng extract (KRG) has a suppressive potency on progression of AFL in an animal model or not, prior to clinical application.

## MATERIALS AND METHODS

**Animals and the Experimental Design** —— Extract form of KRG was obtained from the Korea Ginseng Corporation ("Cheong Kwan Jang", a 6-year-old Korean red ginseng root extract, with crude saponin contents > 70 mg/g, assayed by government laboratory in Daejeon city, Republic of Korea). Forty male Sprague-Dawley rats weighing 180–190 g were purchased from Central Laboratory Animal Incorporation (Seoul, Republic of Korea), divided into four groups and housed in plastic cages at 23–25°C with a 12 hr light/dark cycle. The experimental protocol was approved by the Animal Care and Use Committee of Hanyang University (Seoul, Republic of Korea). Experimental rats were fed a liquid diet containing 95% ethanol which provided 36% of the total energy intake for six weeks, while naive rats were supplied an iso-caloric control diet according to the protocol of Lieber and DeCarli.[21] Dietary energy intake of naive rats were limited to the average intake of the experimental rats. KRG extract was orally administered to the experimental rats at the concentration of 0 (Ethanol + KRG 0 mg/kg group, $n = 10$), 700 (Ethanol + KRG 700 mg/kg group, $n = 10$) or 1400 mg/kg body weight (Ethanol + KRG 1400 mg/kg group, $n = 10$), once a day, five times per week, for six weeks. Naive rats (naive group, $n = 10$) were given isovolumetric distilled water in the same manner, and body weight (g) was also measured every week. At the end of the experimental period, all the rats were anesthetized with ether following a 12 hr fast. Plasma was derived by centrifugation (3000 rpm, 15 min, 4°C) of blood samples obtained by heart puncture. Livers were removed from the body and rinsed with normal saline solution. Then the sliced large lobes were fixed in 10% neutral buffered formalin solution, while the remaining liver tissues were stored at −70°C for further examinations. Liver/body weight (%) was calculated by "(liver (g)/body (g)) × 100."

**Histopathological Examination of Livers** —— Fixed liver tissues were paraffin-embedded and sliced as thick as 4 μm. The sliced specimens were Hematoxyline & Eosin (H&E) stained and observed by light microscopy (× 200) for the evaluation of steatosis, inflammation and necrosis. Hepatic steatosis was evaluated by Tsutsumi and Takase's method[22] which is described as the percentage of hepatocytes containing fat, such as, no fatty degradation (grade 0), one third of hepatic lobules (grade 1), two thirds of the hepatic lobules (grade 2) and the whole of the hepatic lobules (grade 3). Hepatic inflammation and necrosis were evaluated by the presence and the distribution of the neutrophil and necrotic cell infiltration[23] which is described as absent (grade 0, normal), scattered (grade 1, mild), focal (grade 2, moderate) and diffuse (grade 3, severe). The sliced specimens were also Masson's Trichrome (MT) stained and observed by light microscopy (× 200) for the evaluation of fibrosis. Hepatic fibrosis was evaluated by Knodell's scoring system[24] which is described as 'no fibrosis (grade 0),' 'fibrous pericentral expansion (grade 1),' 'bridging fibrosis (grade 2),' 'fibrosis septa and structural disturbance of hepatic lobule (grade 3)' and 'cirrhosis (grade 4).' The pathological alterations of both H&E and MT stained specimens were evaluated and confirmed by a certified independent pathologist.

**Liver Function Tests and Lipid Concentrations of Livers and Plasma** —— Thawed liver tissues were homogenized in chloroform-methanol solution (2 : 1, v/v) in ice-bath, and then centrifuged at 3000 rpm, 20 min, 4°C. The lower chloroform fraction of the homogenized tissue was separated and evaporated by nitrogen gas, then mixed vigorously with chloroform again and evaporated by nitrogen gas for purification.[25] The purified lipids were dissolved by chloroform-Triton X100 solution (19 : 1, v/v) and then analyzed by enzymatic spectrophotometry using commercial kits for triglyceride (Cleantech TG-S, Asan Pharm., Ltd., Hwasung city, Korea) and total cholesterol (T-CHO, Asan Pharm., Ltd.) at 550 and 500 nm, respectively.[26] Plasma total cholesterol and triglyceride levels were determined using Olympus Analyzer (AU400, Olympus Corporation, Tokyo, Japan), and plasma high density lipoprotein (HDL) cholesterol was determined by an enzymatic assay (HBi Co., Ltd., Anyang city, Korea). Low density lipoprotein (LDL) cholesterol was calculated from the equation of "LDL cholesterol = (Total cholesterol − HDL cholesterol − triglyceride)/5; unit = mg/dl)." Con-

**Table 1.** Histopathological Evaluation of Steatosis, Inflammation, Necrosis, Fibrosis and Total Liver Scores in the H&E and MT Stained Liver Tissues of the AFL Rat Model

| | Naive | Ethanol + | | | *p*-value |
|---|---|---|---|---|---|
| | | KRG 0 mg/kg | KRG 700 mg/kg | KRG 1400 mg/kg | |
| Steatosis (%) | $21.24 \pm 2.84^{a*}$ | $48.35 \pm 2.66^{b}$ | $37.22 \pm 3.16^{c}$ | $35.36 \pm 4.38^{c}$ | 0.000 |
| Steatosis | $1.20 \pm 0.13^{a}$ | $2.00 \pm 0.00^{b}$ | $1.67 \pm 0.17^{bc}$ | $1.38 \pm 0.18^{ac}$ | 0.002 |
| Inflammation | $0.90 \pm 0.10^{a}$ | $1.60 \pm 0.16^{b}$ | $1.20 \pm 0.13^{ab}$ | $1.40 \pm 0.16^{b}$ | 0.010 |
| Necrosis | $1.10 \pm 0.23^{a}$ | $2.40 \pm 0.16^{b}$ | $2.40 \pm 0.16^{b}$ | $2.20 \pm 0.20^{b}$ | 0.000 |
| Fibrosis | $0.40 \pm 0.16^{a}$ | $1.00 \pm 0.00^{b}$ | $1.00 \pm 0.00^{b}$ | $0.56 \pm 0.18^{a}$ | 0.001 |
| Total scores | $3.30 \pm 0.26^{a}$ | $6.60 \pm 0.40^{b}$ | $6.10 \pm 0.35^{cb}$ | $5.20 \pm 0.47^{c}$ | 0.000 |

Mean ± S.E. Steatosis (0–3), inflammation (0–3), necrosis (0–3), fibrosis (0–4) and total liver scores (0–13). * Each group which has different superscript letters is significantly different each other in each row by ANOVA following Duncan post-hoc test at $p < 0.05$.

centrations of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in plasma were also determined to evaluate liver function using respective kits from Asan Pharm., Ltd.

**Hydroxyproline Contents in Livers** —— For the evaluation of liver fibrosis and collagen accumulation, hydroxyproline was determined in the liver tissues according to the modified method of Reddy and Enwemeka.[27] Thawed liver tissues were homogenized in 6 N hydrochloric acid solution and hydrolyzed in the autoclave at 121°C, 20 min. After cooling them to room temperature, the upper fractions of the liver hydrolysates, in triplicate, were added to separate vials, and allowed to evaporate to dryness under nitrogen gas. The residues were dissolved in 50% isopropanol, with added 6.7% Chloramin T and Ehrlich's solutions, then incubated at 60°C, for 20–90 min. After rapid cooling them in cold water, hydroxyproline was assayed using spectrophotometer at 558 nm.

**$^{201}$Thallium (Tl) per Rectum Scintigraphy** —— To evaluate the portal pressure as a prognostic indicator of portal systemic shunt, $^{201}$Tl per rectal scintigraphy was performed in the rats according to the procedure described by Tonami[28] and Urbain.[29] At the end of the experiment, rats were fasted for 12 hr and then administered sodium phosphate enema through the rectum for 40–60 min under ether-anesthesia. After emptying the colon of the rats, heart/liver radioactivity ratio of $^{201}$Tl (H/L ratio) was detected using Nuclear Gamma Camera Siemens orbiter 7500 (Siemens medical solutions U.S.A. Inc., Malvern, PA, U.S.A.) equipped with low energy all purpose single head collimator at the site of liver and heart regions after 18–20 minutes of intrarectal administration of 0.5 mCi $^{201}$Tl. An increased H/L ratio implies portal pressure change caused by liver fibrosis.

**Statistical Analysis** —— All the measurements were presented as mean ± S.E., and statistical significance among the groups was analyzed by analysis of variance (ANOVA) following Duncan post-hoc test at $p < 0.05$ using SPSS version 17.0 program. Only weight data were analyzed by repeated measured ANOVA through the study period at $p < 0.05$.

## RESULTS

### Effects of KRG on Histopathological Characteristics in Livers

Histopathological analysis by H&E and MT staining showed significant development of micro- or macro-vesicular steatosis, as well as necrosis accompanied by inflammatory cell infiltration in the ethanol administered rats over the six weeks period (Fig. 1). Among the ethanol administered rats, co-administration of KRG attenuated the deposition of fat effectively, and diminished inflammatory cells infiltration and necrosis in livers mildly, while the worst evidence of liver injury was exhibited accompanying the greatest degree of fat accumulation in 1/3–2/3 of the hepatic lobules, scattered or focal neutrophils and necrotic cells in liver tissues without co-administration of KRG (Fig. 1, H&E). The percentage of hepatosteatosis was evaluated as 21.24% for naive rats, and it was increased in the order of Ethanol + KRG 1400 mg/kg (35.36%), Ethanol + KRG 700 mg/kg (37.22%) and Ethanol + KRG 0 mg/kg (43.35%) groups ($p = 0.000$, Table 1). Fibrous pericentral expansion was also noticed in the Ethanol + KRG 0 mg/kg or Ethanol + KRG 700 mg/kg groups ($p =$

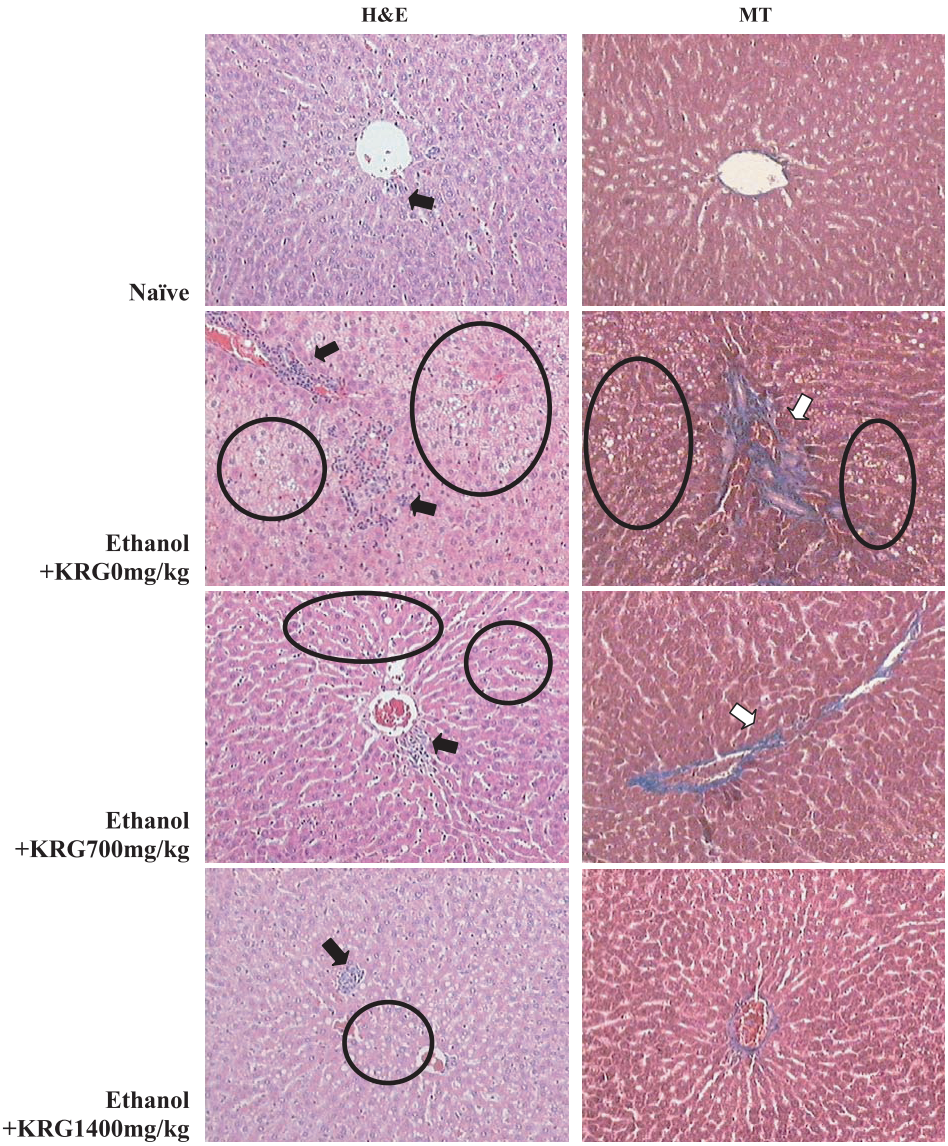


**Fig. 1.** Histopathological Findings of H&E Stained or MT Stained Livers in the AFL Rat Model (×200)

Black ovals indicate macrovesicular steatosis and black circles indicate microvesicular steatosis. Black arrows indicate inflammatory cell infiltration with necrosis and white arrows indicate fibrotic changes.

0.001, Fig. 1, MT and Table 1). Overall anti-hepatosteatotic effect was stronger when KRG 1400 mg/kg was administered to the ethanol-induced rats, as the total liver score ($p$ = 0.000) which includes steatosis ($p$ = 0.002), inflammation, necrosis and fibrosis ($p$ = 0.001) exhibited (Table 1).

### Effects of KRG on Hydroxyproline Contents in Livers and H/L ratios by $^{201}$Tl per Rectal Scintigraphy Test

Hepatic hydroxyproline contents are index of collagen accumulation which is a key component of the fibrotic tissues observed in the alcoholic liver diseases. The hepatic hydroxyproline contents were significantly elevated after ethanol consumption, however co-administration of KRG 700 or 1400 mg/kg depressed mildly the deposit of hydroxyproline in livers (Table 2, $p$ = 0.021). H/L ratios by $^{201}$Tl per rectal scintigraphy were not significantly changed by the administration of ethanol or KRG for six weeks, even though Ethanol + KRG 0 mg/dl and Ethanol + KRG 700 mg/dl groups had relatively higher H/L ratios than naive and Ethanol + KRG 1400 mg/kg groups' (Table 2).

### Effects of KRG on Hepatic and Plasma Lipid Concentrations, and Liver Function Markers

**Table 2.** Hydroxyproline Contents (μg/g) in Livers and H/L Ratios by $^{201}$Tl per Rectal Scintigraphy in the AFL Rat Model

| | Naive | Ethanol + | | | *p*-value |
|---|---|---|---|---|---|
| | | KRG 0 mg/kg | KRG 700 mg/kg | KRG 1400 mg/kg | |
| Hydroxyproline | 279.50 ± 17.78$^{a*}$ | 345.42 ± 10.84$^{b}$ | 308.88 ± 16.72$^{ab}$ | 315.00 ± 8.50$^{ab}$ | 0.021 |
| H/L ratio | 0.187 ± 0.022 | 0.227 ± 0.024 | 0.229 ± 0.020 | 0.199 ± 0.013 | 0.380 |

Mean ± S.E. * Each group which has different superscript letters is significantly different each other in each row by ANOVA following Duncan post-hoc test at $p < 0.05$.

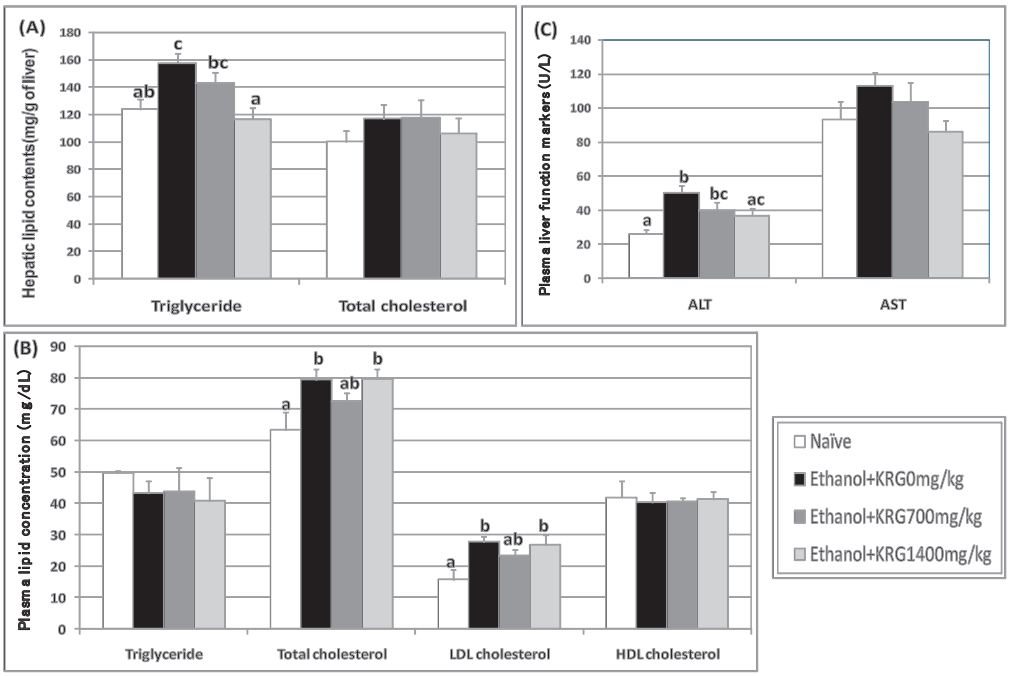


**Fig. 2.** Comparisons of Hepatic Lipids, Plasma Lipids and Liver Function Markers in Plasma

Hepatic lipid contents (A), plasma lipid concentrations (B) and liver function markers (C) in the AFL rat model (mean ± S.E.). Each group which has different alphabetical letters such as a, b, and c is significantly different each other by ANOVA following Duncan post-hoc test at $p < 0.05$.

The contents of triglyceride in livers (Fig. 2A) were significantly increased in ethanol administered rats, however co-administration of KRG 700 or 1400 mg/kg suppressed the hepatic accumulation of triglyceride ($p = 0.003$). Specially, AFL was remarkably attenuated to the level of naive rats by co-administration of higher concentration of KRG (1400 mg/kg). Hepatic contents of total cholesterol (Fig. 2A) had a similar trend of triglyceride contents, even though it was not significantly. The plasma total cholesterol and LDL cholesterol levels (Fig. 2B) were significantly increased in ethanol administered rats, however, co-administration of KRG 700 mg/kg suppressed the elevation of total cholesterol and LDL cholesterol levels in plasma mildly ($p = 0.015$ and 0.011, respectively). Plasma triglyceride and HDL cholesterol concentrations (Fig. 2B) were not significantly influenced by ethanol or KRG administration during the six-week experiment. Representative markers of liver function were presented in Fig. 2C, and concentration of plasma ALT was significantly increased in ethanol administered rats ($p = 0.002$). However, co-administration of KRG prevented the elevation of ALT level in plasma, and the preventive effect was more significant when co-administered KRG 1400 mg/kg ($p = 0.002$). Plasma AST level showed a same trend of ALT level, even though it was not significant enough.

## Effects of KRG on Liver/Body Weight Percentages

The body weights of the rats (Fig. 3A) were significantly increased after two weeks of the experiment when the time went by ($p < 0.05$ each). The body weight each week of the naive rats (Fig. 3A)

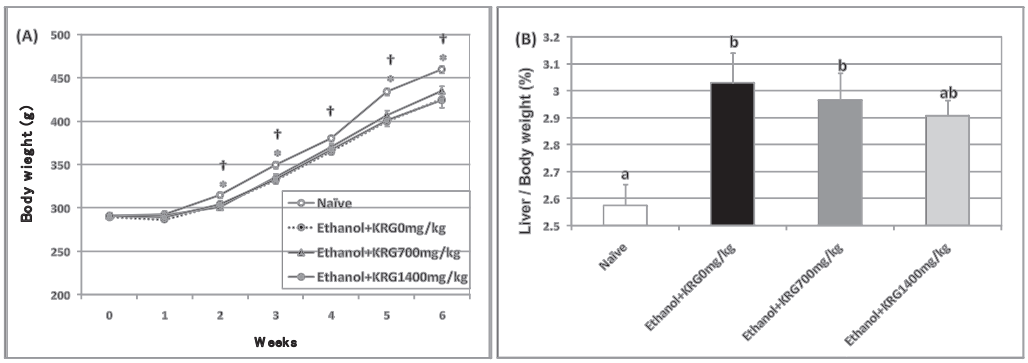


**Fig. 3.** Comparisons of Body Weight and Liver to Body Weight Percentages

Variations of body weight for six weeks (A) and liver to body weight percentages (B) in the AFL rat model (mean ± S.E.). A cross (†) indicates that mean body weight of the week is different from the weight of initial week at $p < 0.05$, and an asterisk (∗) indicates that mean body weight of naive group is different from the ethanol administered groups' each week at $p < 0.05$. Different letters mean the statistical differences of liver/body weight (%) among the groups by ANOVA following Duncan post-hoc test at $p < 0.05$. Each group which has different alphabetical letters such as a and b is significantly different each other in its liver/body weight (%) by ANOVA following Duncan post-hoc test at $p < 0.05$.

was significantly heavier than the ethanol administered rats at the second, third, fifth and sixth weeks ($p < 0.05$ each). However, body weights were not different among the ethanol administered rats. On the contrary, the liver to body weight percentages of the ethanol administered rats (Fig. 3B) were significantly higher than the naive rats, and the co-administration of KRG 1400 mg/kg could mildly attenuate the excessive liver mass ($p < 0.05$).

## DISCUSSION

AFL, the initial stage of alcoholic liver diseases, is marked by fatty metamorphosis of hepatocytes which accumulate in the liver and which are highly sensitive to further alcoholic insult leading to the development of more severe manifestations of alcohol-induced liver diseases such as hepatitis, fibrosis and finally, cirrhosis.[30, 31] In the current study, interventional administration of the herbal medicine, KRG, had a magnificent anti-hepatosteatotic effect in AFL rat model when it was orally administered for six weeks, and the protective effect was proved by evaluation of histopathological characteristics (Fig. 1, H&E and Table 1), hepatic lipid contents (Fig. 2A) and liver/body weight (Fig. 3B). The anti-hepatosteatotic effect was so dose-dependent that KRG 1400 mg/kg was more beneficial than KRG 700 mg/kg. In addition to the anti-hepatosteatotic effect, anti-inflammatory effect of KRG on AFL was also noticed (Fig. 1A and Table 1). It was explained by dose-dependent decreases of plasma ALT ($p < 0.05$) and AST levels which represented hepatocyte and mitochondrial injuries in livers, respectively (Fig. 2C), even though hepatic mitochondrial function was not distinctly altered during the six-week study. Fibrotic changes associated with more advanced stages of alcoholic liver diseases were also significantly retarded by KRG co-administration (Fig. 1, MT and Table 1), and it was supported by reduction of hepatic hydroxyproline contents, which represents hepatic collagen formation, in KRG co-administered rats (Table 2). Even though the severity of fibrosis was not serious enough to elevate the hepatic portal pressure (H/L ratio) during the six-week of alcohol intake, it was meaningful that initiation of fibrotic changes from AFL could be suppressed by administration of KRG (Table 2). H/L ratios at 20 min after administration of $^{201}$Tl were reported that $> 0.16$ from 'normal livers without alcoholic damages,' $> 0.25$ from 'chronic hepatitis,' $> 0.40$ from 'alcoholic fatty liver without cirrhosis' and $> 0.92$ from 'cirrhosis with diverse hepatocellular damages and significant differences.'[28, 32]

Interestingly, plasma triglyceride and HDL cholesterol levels were not altered by ethanol or KRG administration either, and plasma total cholesterol and LDL cholesterol levels were improved when KRG 700 mg/kg were co-administered in the current study (Fig. 2B). These plasma lipid patterns after alcohol consumption were very similar to other's six-week study results.[33] However, Horie *et al.*[34] reported that plasma triglyceride and total cholesterol concentrations increased after alco-

hol consumption. The partial inconsistancy between peripheral and hepatic lipid concentrations in the current study might be caused by the easily fluctuated biochemical characteristics of plasma, while relatively stable characteristics of liver tissue. This inconsistency could be corrected by prolongation of the study period over two or three months, resulting in more correlated and dose-dependent results between liver and plasma when administered ethanol or KRG.[35)]

Nevertheless plasma lipid profiles were not dose-dependent to KRG co-administration, anti-hepatosteatotic effect of KRG on AFL rat model was proved dose-dependently by the examinations of liver lipid contents, histopathological findings, liver to body weight and liver function tests. How AFL could be improved by ingestion of KRG 700–1400 mg/kg per day was not fully understood by this study, however we referred that KRG prevented or retarded alcoholic disruption of lipid metabolism in livers, reflected by changes in the hepatic lipid, possibly lipoproteins, metabolism such as impairment of transportation of lipoproteins,[36)] inhibition of fatty acid oxidation,[37)] and enhancement of lipogenesis,[38)] as well as other toxic effects on hepatic function such as AST and ALT levels in plasma caused by acetaldehyde.[37)] Energy intake[39)] and fatty acid composition of the diet[40)] could also influence the development of AFL, however energy derived from ethanol in the diet were strictly controlled energy-proportionally by pair-fed matching with corn-starch among the groups in this study. Dietary energy inputs from polyunsaturated fatty acids like corn oil and safflower oil were constant among the groups at 8.5 and 2.7% of total energy intakes respectively, and contributions of monounsaturated fatty acids like olive oil were held at 28.4% of total dietary input. These low compositions of polyunsaturated fatty acid consumption could not influence the induction of AFL. According to the control of energy intakes, body weight variations along the time sequences were not significantly different among the ethanol-administered rats, however naive rats were distinctly heavier than the ethanol-administered rats (Fig. 3A). This difference might be caused by wasteful expenditure of energy under chronic consumption of alcohol, and by less effectively deposited energy as body mass from alcohol than that from carbohydrate,[41)] resulting in impairment of lipid metabolism in livers.

Additionally, the dose of KRG 700–1400 mg/kg for suppressing AFL in this study seemed quite high. However it is acceptable as a pharmacodynamic dose for animal studies when considered that a recommendation of Korea Ginseng Corporation for KRG is 3 g/day for adults as a food supplement in Republic of Korea, and that KRG has relatively small amount (about 70 mg/g of KRG) of saponin which is a representative effective component. Besides, KRG was less toxic than fresh Korean ginseng.[13, 14)] In other studies, Kim and Park[42)] examined the effect of Panax ginseng extract in humans at the high dose of 6 g/day for eight weeks without any reported toxicity. And Kwak *et al.*[43)] examined also the effect of high dose (100–1000 mg/kg) of red ginseng acidic polysaccharide in a rat model, resulting in significant anti-hyperlipidemic effect at 1000 mg/kg dose.

In conclusion, co-administration of KRG significantly retarded development of AFL and prevented development of early signs of the progression to cirrhosis at the oral dose of KRG 1400 mg/kg per day for six weeks. To elucidate possible mechanisms for the protective effects of KRG on steatosis, inflammation and fibrosis in the alcoholic liver disease, further studies are needed to examine hepatic function and toxicity by redox changes and oxidative stress with extended study period.

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